

AWARD NUMBERS: W81XWH-14-1-0519

TITLE: Development of a New Class of Drugs to Inhibit All Forms of Androgen Receptor in Castration-Resistant Prostate Cancers

PRINCIPAL INVESTIGATOR: Paul Rennie

CONTRACTING ORGANIZATION: University of British Columbia  
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**14. ABSTRACT**

Prostate cancer is the most frequently diagnosed male cancer and second leading cause of male cancer death. Management of patients with advanced-stage disease relies on inhibiting the androgen receptor (AR) with conventional endocrine targeting therapies, and more recently with second-generation endocrine targeting therapies designed to block AR activity that re-emerges during castration. However, despite a growing armamentarium of drugs targeting the androgen/AR signaling axis, progression of castration-resistant prostate cancer (CRPC) remains a major clinical challenge that undermines survival and quality of life for prostate cancer patients. The proposed research is focused on the pre-clinical development of VPC14228, a drug-like small molecule that targets the AR:DNA interaction. During the first year of this award, we have made progress in investigating the functional effects of VPC14228 on DNA interaction and transcriptional activation mechanisms for AR, developing a definitive experimental and structural characterization of the VPC14228 interaction with the AR DBD, and conducting pre-clinical evaluation of VPC14228.

**15. SUBJECT TERMS**

Nothing listed

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## **1. INTRODUCTION**

Prostate cancer is the most frequently diagnosed male cancer and second leading cause of male cancer death. Management of patients with advanced-stage disease relies on inhibiting the androgen receptor (AR) with conventional endocrine targeting therapies, and more recently with second-generation endocrine targeting therapies designed to block AR activity that re-emerges during castration. However, despite a growing armamentarium of drugs targeting the androgen/AR signaling axis, progression of castration-resistant prostate cancer (CRPC) remains a major clinical challenge that undermines survival and quality of life for prostate cancer patients. The proposed research is focused on the pre-clinical development of VPC14228, a drug-like small molecule that targets the AR:DNA interaction. The goals of this research are to investigate the functional effects of VPC14228 on DNA interaction and transcriptional activation mechanisms for AR, develop a definitive experimental and structural characterization of the VPC14228 interaction with the AR DBD, and conduct pre-clinical evaluation of VPC14228.

## **2. KEYWORDS**

Androgen receptor, castration-resistant prostate cancer, DNA binding domain, androgen response element, AR inhibitor, chromatin, x-ray crystallography, pre-clinical

## **3. ACCOMPLISHMENTS**

### **a. Major goals of the project as outlined in the approved Statement of Work (SOW):**

- 1.1. Assay luciferase reporters in prostate cancer cell lines (months 1-12).
- 1.2. Determine effects of VPC14228 on AR binding to genomic AREs (months 13-24)
- 1.3. Chromatin immunoprecipitation (ChIP) assays with lentivirus-infected cells to test AR-V binding to genomic AREs (months 13-24)
- 1.4. Obtain approval for mouse xenograft studies from U of M IACUC and USAMRMC ACURO (months 1-6).
- 1.5. Establish xenografts with prostate cancer cell lines to generate tissue for chromatin fractionation and ChIP-seq assays (months 7-36)
- 1.6. Perform chromatin fractionation assays with prostate cancer cell lines and xenografts (months 1-6).
- 1.7. Perform ChIP-seq with prostate cancer cell lines and xenografts (months 7-36).
- 2.1. Form crystals of ARdbd in complex with ARE DNA (Months 1-6).
- 2.2. Optimize ligand soaking conditions (Months 7-18).
- 2.3. Collect native and Zn- or Br-anomalous X-ray diffraction data from VP14228-soaked crystals using synchrotron radiation (Months 18-21).
- 2.4. Obtain phases by molecular replacement and single wavelength anomalous dispersion (Months 18-21).
- 2.5. Identify the ligand and its binding site, build the VP14228-ARdbd-DNA model, and refine the structure (Months 21-24).
- 2.6. Crystallize ARdbd or ARtnd in the presence and absence of VP14228 without DNA (Months 1-20).
- 2.7. Soak VP14228 into apo ARdbd or ARtnd crystals (Months 12-20).

- 2.8. Collect native and Zn-anomalous X-ray diffraction data from co-crystallized, soaked, and apo ARdbd or ARtnd crystals, with the final data sets collected using synchrotron radiation (Months 20-24).
- 2.9. Obtain initial phases by molecular replacement and single wavelength anomalous dispersion (Months 24-26).
- 2.10. Build the model and refine the structure (Months 26-30).
- 3.1. Molecular modeling of derivatives of our current lead VPC-14228 (months 1-30)
- 3.2. Synthesis of derivatives of our lead compounds (months 6-30).
- 3.3. Experimental evaluation of the developed synthetic derivatives (months 4-30)
- 3.4. Selection of several lead compounds for pharmacological development

## **b. Accomplishments under these goals:**

### **Major Activities for University of Minnesota Site (Dehm, PI)**

In the first reporting period, the major activities consisted of a) Testing the effects of VPC14228 and the derivative VPC14449 on AR transcriptional activity in prostate cancer cell lines using luciferase reporter assays; b) Testing the effects of VPC14228 and the derivative VPC1449 on AR chromatin binding and transcriptional output in prostate cancer cell lines using ChIP and quantitative RT-PCR approaches; c) Testing the effects of VPC14228 on AR-V chromatin binding in prostate cancer cell lines using lentivirus infection and ChIP; d) applying for and securing approval from IRB and ACURO authorities to perform mouse studies; e) Testing the effects of VPC14228 and the derivative VPC14449 on AR chromatin binding using chromatin fractionation techniques.

### **Major Activities for Hauptman Woodward Institute Site (Gewirth, PI)**

In the second reporting period, the major activities consisted of a) expressing and purifying large quantities of the ARdbd protein, b) expressing and purifying human ARdbd protein; c) expressing and purifying the K575A mutant of rat ARdbd protein; d) growing crystals of rat ARdbd in the presence of VP14449; e) forming the human ARdbd-DNA complex; f) carrying out crystallization procedures and trials on the human ARdbd-DNA complex; g) carrying out isothermal titration calorimetry studies between rat ARdbd protein and VP14449.

### **Major Activities for Vancouver Prostate Centre Site (Rennie, PI)**

In the previous reporting period, we began work to a) Determine the efficacy of VPC-14449 to suppress AR transcriptional activity in different prostate cancer cell lines that model drug-resistance, b) to explore the ability of VPC-14449 to downregulate target genes in these cell-lines, and c) to determine the ability of VPC-14449 to inhibit a diverse array of mutations in the androgen receptor.

### **Specific Objectives for University of Minnesota Site (Dehm, PI)**

The specific objectives for this reporting period were to a) Determine effects of VPC14228 on AR binding to genomic AREs, b) perform chromatin immunoprecipitation (ChIP) assays with lentivirus-infected cells to test AR-V binding to genomic AREs, c) establish xenografts with prostate cancer cell lines to generate tissue for chromatin fractionation and ChIP-seq assays, d) perform chromatin fractionation assays with prostate cancer cell lines and xenografts e) perform

ChIP-seq with prostate cancer cell lines and xenografts (months 7-36). These objectives correspond to Tasks 1.2, 1.3, and 1.5-1.7 as described in the approved SOW.

**Specific Objectives for Hauptman Woodward Institute Site (Gewirth, PI)**

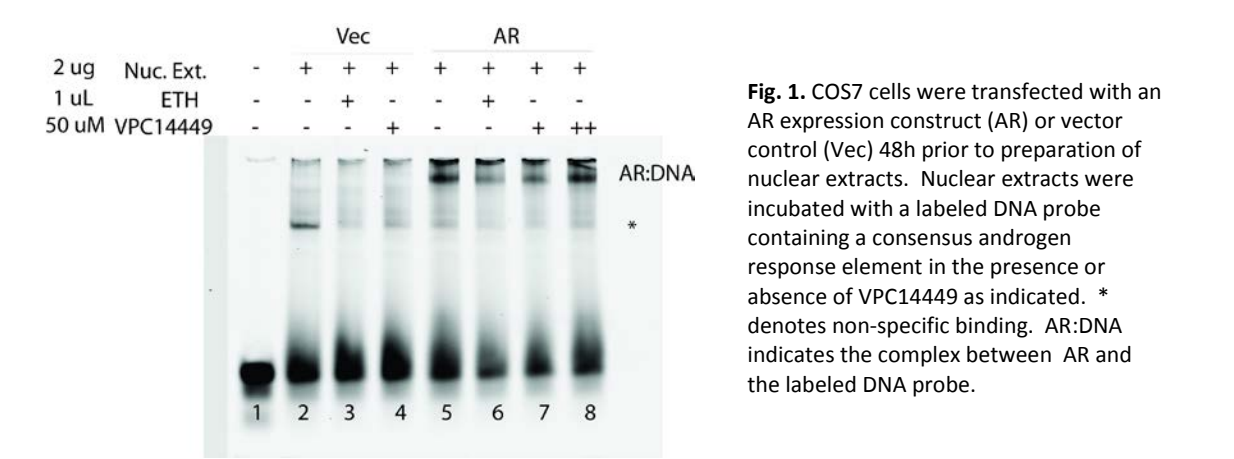
The specific objectives for this reporting period were to a) produce ARdbd protein from both rat and human, b) form human ARdbd-DNA complexes, c) grow crystals of the ARdbd alone or in complex with VP14449, d) quantitate the binding of VP14449 to ARdbd. These objectives correspond to Tasks 2.1, 2.2, 2.6, and 2.7 as described in the approved SOW.

**Specific Objectives for Vancouver Prostate Centre Site (Rennie, PI)**

The specific objectives for the Rennie/Cherkasov labs in this reporting period were to a) model and synthesize derivatives of VCP-14449, b) form a strategic partnership with Industry (Roche Pharmaceuticals) to accelerate the development of better and more metabolically stable inhibitors, and c) To experimentally evaluate the new inhibitors against our pipeline of biochemical and cellular validation techniques. These objectives pertain to tasks 3.1.-3.4 in the SOW.

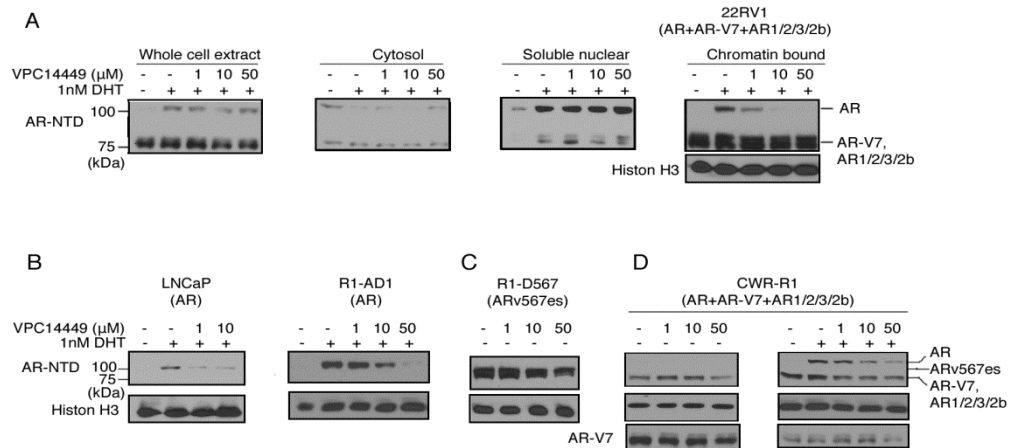
**Key Outcomes for University of Minnesota Site (Dehm, PI)**

Key Outcome 1: VPC14449 inhibits binding of AR and AR variants to chromatin *in vivo*. We used electrophoretic mobility shift assays to test the effect of VPC14449 on AR binding to DNA *in vitro*. In nuclear extracts prepared from COS7 cells transfected with an AR expression construct, VPC14449 has no effect on AR binding to an androgen response element (ARE) (Fig. 1).



**Fig. 1.** COS7 cells were transfected with an AR expression construct (AR) or vector control (Vec) 48h prior to preparation of nuclear extracts. Nuclear extracts were incubated with a labeled DNA probe containing a consensus androgen response element in the presence or absence of VPC14449 as indicated. \* denotes non-specific binding. AR:DNA indicates the complex between AR and the labeled DNA probe.

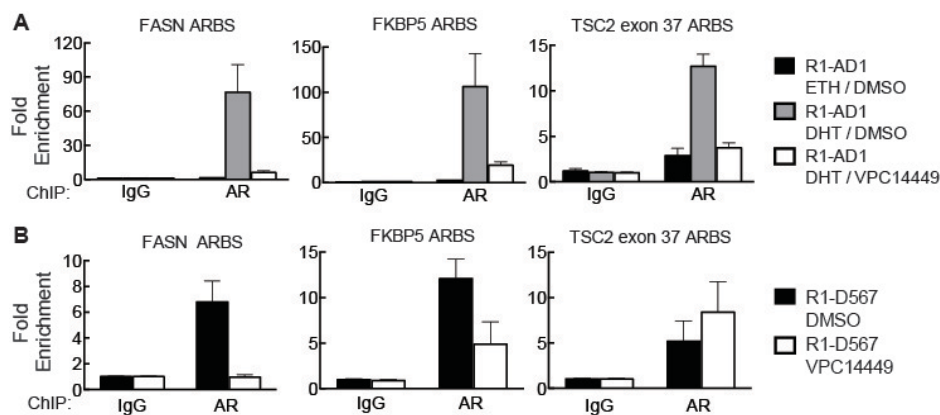
We next used subcellular fractionation techniques to test the effects of VPC14449 on AR and AR variant (AR-V) expression, subcellular localization, and binding to chromatin AREs *in vivo*. VPC14449 did not inhibit expression levels of AR or AR-Vs, and did not affect the ability of these species to translocate to the nucleus (Fig. 2A). However, VPC14449 inhibited chromatin binding of full-length AR and AR-Vs, although the effect on full-length AR was more pronounced (Fig. 2A). Similarly, VPC14449 inhibited chromatin binding of AR and AR-Vs in additional models of full-length AR expression (Fig. 2B), AR-V expression (Fig. 2C), or mixed AR/AR-V expression (Fig. 2D).



**Fig. 2. (A)** 22RV1 cells were treated with dihydrotestosterone (DHT) and VPC14449 as indicated, and subjected to subcellular fractionation to prepare whole cell extracts as well as cytosolic, soluble nuclear, and insoluble nuclear (chromatin) fractions. Cell fractions were subjected to western blot with an antibody specific for the AR NTD. Chromatin fractions from **(B)** LNCaP and R1-AD1 cells expressing full-length AR, **(C)** R1-D567 cells expressing AR v567es, and **(D)** CWR-R1 cells expressing AR, AR-V7 and AR 1/2/3/2b were subjected to western blot with an antibody specific for the AR NTD as in A.

### Key Outcome 2: VPC14449 inhibits binding of AR and AR variants to discrete AREs *in vivo*.

To test the effects of VPC14449 on the ability of AR and AR-Vs to bind discrete AREs, we performed chromatin immunoprecipitation (ChIP) with antibodies specific for the AR NH2-terminal domain (NTD). VPC14449 inhibited full-length AR binding to AREs in the FASN, FKBP5, and TSC2 loci (Fig. 3A). VPC14449 also inhibited ARv567es binding to AREs in the FASN and FKBP5 loci, but had no effect on ARv567es binding to the TSC2 ARE (Fig. 3B). This aligns with chromatin fractionation data (Fig. 2), supporting the concept that VPC14449 inhibits binding of AR-Vs to select AREs *in vivo*.



**Fig. 3. (A)** R1-AD1 cells expressing full-length AR were subjected to ChIP to test the impact of VPC14449 on AR binding to AREs in the FASN, FKBP5, and TSC2 loci. **(B)** R1-D567 cells expressing ARv567es were subjected to ChIP to test the impact of VPC14449 on ARv567es binding to AREs in the FASN, FKBP5, and TSC2 loci.



## Key Outcomes for Hauptman Woodward Institute Site (Gewirth, PI)

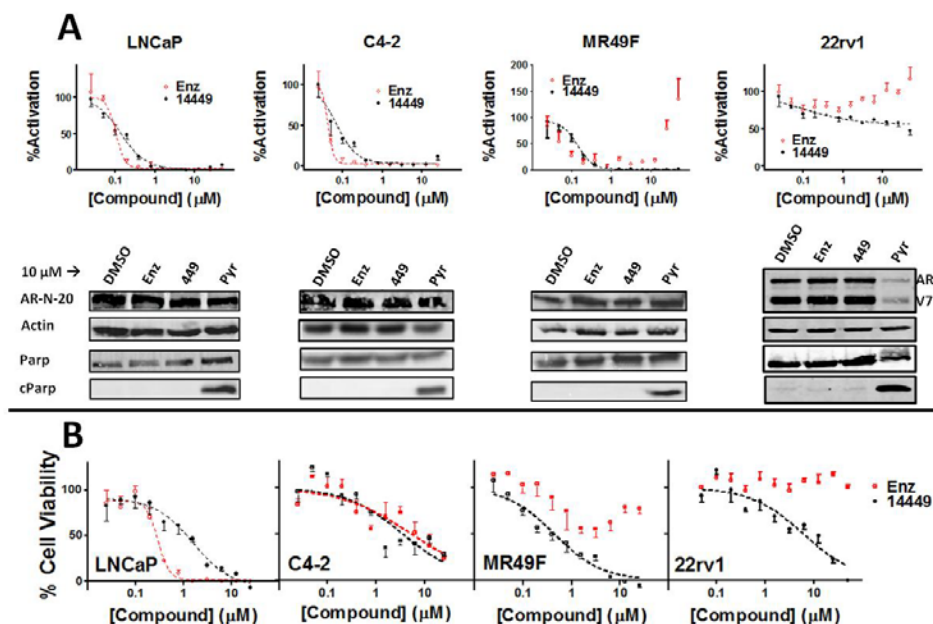
Key outcome 1: We have successfully produced both rat and human ARdbd protein, the rat and human ARdbd-DNA complexes, and have grown crystals of human ARdbd in complex with DNA. The crystals are morphologically different from our previous rat ARdbd-DNA crystals and may represent a new crystal form.

Key outcome 2: We have carried out followup crystallization screening operations with the ARdbd protein in complex with VP14449 ligand. Further optimization is necessary in order to obtain diffraction quality crystals.

Key outcome 3: We have carried out extensive isothermal titration calorimetry (ITC) analysis of the binding of VP14449 to rat ARdbd. Using reverse titration methods (protein in the syringe, ligand in the cell), we were able to obtain a reproducible thermogram for the ligand binding reaction. The calculated dissociation constant from these experiments depends on the method of analysis but is consistent with a  $K_d$  of between 60 and 400 micromolar. This data provides the first biophysical proof for the VP14449:ARdbd interaction, a major milestone in this project.

## Key Outcomes for Vancouver Prostate Centre Site (Rennie, PI)

Key Outcome 1: VPC-14449 inhibits AR-transcriptional activity and cell viability in drug-resistant PCa cell lines. VPC-14449 was tested in transcriptional activation and cell growth assays using various cell lines that express mutated versions of the AR (LNCaP, C4-2, MR49F) or truncated variants (22rv1). It was shown that the compound is effective to varying degree in every cell line tested (**Fig. 4**), indicating the bypass of drug-resistance mechanisms.

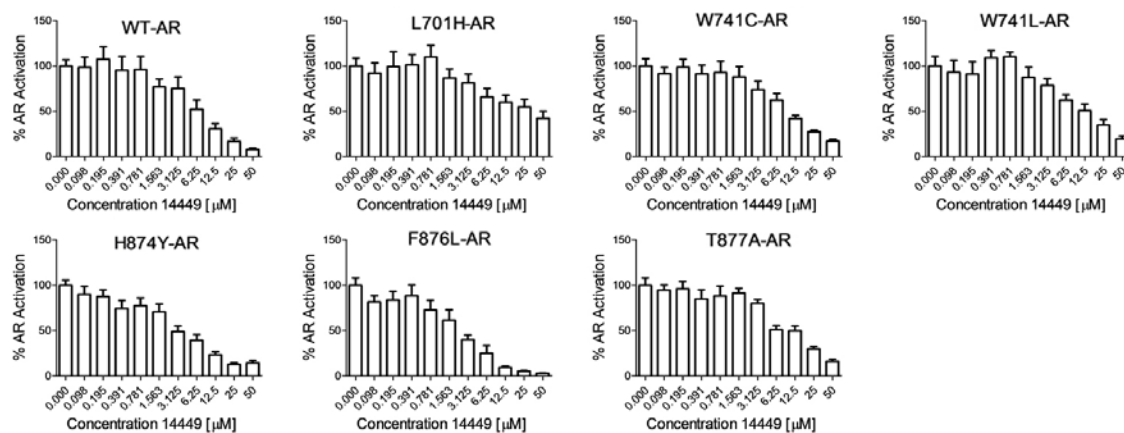


**Fig. 4. (A)** The indicated cell-lines were seeded (96 well plates, RPMI+CSS media) and co-transfected with ARR<sub>3</sub>tk-Luc (50 ng/well) and Renilla-Luc (2 ng/well) plasmids (48 hours). With the exception of 22rv1 cells, 0.1 nM R1881 was used to stimulate the endogenously produce AR. Either enzalutamide (Enz) or VPC-14449 were administered (24 hours) at increasing compound

concentration. Error bars represent the mean and SEM of 4 replicates for each concentration and are normalized to constitutive Renilla expression. 100% refers to normalized luminescence in the absence of compounds. Curves were fitted to a sigmoid dose-response and variable slope equation in GraphPad Prism software. Bottom panels in A show western blots of cell lysates (AR-N20/Actin antibodies) after the indicated cell line was treated for 24 hours with 10  $\mu$ M concentration of the indicated compounds or DMSO controls. *Pyr* refers to pyrvinium pamoate, a previously discovered AR-DBD inhibitor with toxic properties. Lysates were also probed with anti-PARP/cleaved PARP antibodies as a measure of apoptosis. **(B)** Cell viability was measured using an MTS assay with the indicated cell-lines, cultured in RPMI+CSS as in A, following 72 hours compound treatment with 1 nM R1881 (except 22rv1 = no R1881 treatment). Error bars represent the mean and SEM of 4 replicate per point where 100% refers to viability without compound treatment.

## Key Outcome 2. VPC-14449 bypasses AR-LBD mutations involved in anti-androgen resistance.

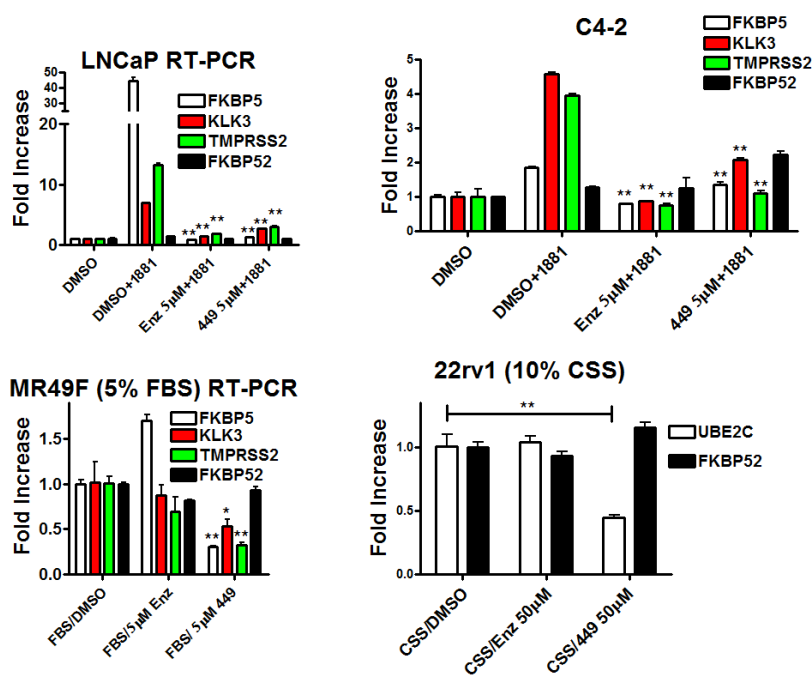
An AR-DBD specific inhibitor should bypass all mutations in the AR ligand binding domain (LBD) that are known to confer enzalutamide or bicalutamide resistance in tumor cells. After sequencing the androgen receptor in circulating free DNA from the blood of actual prostate cancer patients (Lallous et al., 2016, *Genome Biol.*) we selected six additional AR-LBD substitutions that are ostensibly induced from anti-androgen treatment and performed luciferase reporter assays in PC3 cells with transiently expressed AR bearing each individual point mutation. Consistent with the DBD as the site of action, the transcriptional activity of every tested AR-LBD mutant was inhibited by VPC-14449 (**Fig. 5**)



**Fig. 5.** PC3 cells were co-transfected with a plasmid encoding the wild-type (WT) or mutant receptor and ARR<sub>3</sub>tk-Luc reporter (50 ng/well each) and stimulation with 0.1 nM R1881 as described in Fig. 4A. L701H, W741C/L, H874Y, F876L and T877A are all mutations that occur in the AR-LBD. 100% refers to the luminescence recorded in the absence of compounds.

## Key Outcome 3. Down-regulation of full length and variant target-genes by VPC-14449 treatment.

The cell lines used in Fig. 4 were treated with VPC-14449 to determine if AR target-genes could be suppressed (**Fig. 6**). The results show that VPC-14449 suppressed 3 archetypical downstream targets of the AR, including FKBP5, TMPRSS2 and KLK3 (PSA). In 22rv1, high concentration of VPC-14449 suppressed the mitosis gene UBE2C, a known target of ARV7 (Hu et al., 2012, *Cancer Res.*).



**Fig. 6.** PCa cell were cultured with 1 nM R881 and 5 µM compounds where indicated for 24 hours. (A) LNCaP/C4-2 cells (RPMI+CSS) were cultured with or without R881 and the indicated compounds, whereas (B) MR49F cells were cultured in RPMI + 5% FBS (containing natural androgens) and compounds. (C) 22rv1 (RPMI+10% CSS) were treated with 50 µM compound (no R881). RT-PCR was performed to determine relative amounts of the indicate genes and FKBP52 negative control \*=p-value  $\leq 0.05$ , \*\*=p-value  $\leq 0.01$  comparing expression b/w DMSO+R881 and compound+1881 (LNCaP, C4-2), b/w FBS/DMSO and FBS/compounds (MR49F) and b/w CSS/DMSO and CSS/compounds (22rv1) treatment conditions.

### **c. Opportunities for training and professional development:**

Nothing to report

### **d. Results disseminated to communities of interest:**

Nothing to report

### **e. Plans for next reporting period:**

#### **University of Minnesota Site (Dehm, PI)**

In the current reporting period, we have established the optimized treatment conditions for VPC14449 in prostate cancer cell lines models, and verified that VPC14449 inhibits the binding of AR and AR variants to chromatin AREs. In the next reporting period, we will conduct ChIP-seq experiments to understand the genome-wide impact of VPC14449 on AR and AR-V binding to chromatin AREs in prostate cancer cell lines grown *in vitro* and as xenografts *in vivo*.

#### **Hauptman Woodward Institute Site (Gewirth, PI)**

Having established that ARdbd exhibits a binding profile by ITC with VP14449, we will return to our crystallization experiments with the goal of optimizing the ARdbd-VP14449 crystals. We will also continue to pursue the crystallization of the apo ARdbd protein, either alone or as a fusion protein, in order to provide a more robust platform for VP14449 soaking experiments.

### **Vancouver Prostate Centre Site (Rennie, PI)**

Now that the mechanism of action of VPC-14449 has been better characterized, we will attempt to show efficacy in mouse tumor xenografts using MR49F and C4-2 cell lines. Although VPC-14449 has poor pharmacokinetic properties in animals, it should nevertheless be able to suppress tumor growth in these two model cell-lines of drug-resistance. In addition, we will continue working with our industry partner to develop improved versions of VPC-14449. Finally, a manuscript is nearly complete describing the mechanism of VPC-14449 activity drawing together all the new findings from the Rennie/Cherkasov, Dehm and Gewirth Labs.

## **4. IMPACT**

### **a. Impact on the development of the principal discipline(s) of the project?:**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report  
Vancouver Prostate Centre Site: Nothing to Report

### **b. Impact on other disciplines:**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report  
Vancouver Prostate Centre Site: Nothing to Report

### **c. Impact on technology transfer:**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report  
Vancouver Prostate Centre Site: Nothing to Report

### **d. Impact on society beyond science and technology:**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report  
Vancouver Prostate Centre Site: Nothing to Report

## **5. CHANGES/PROBLEMS**

### **a. Changes in approach and reasons for change**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report  
Vancouver Prostate Centre Site: Nothing to Report

### **b. Changes that had a significant impact on expenditures**

University of Minnesota Site: Nothing to Report  
Hauptman Woodward Institute Site: Nothing to Report

Vancouver Prostate Centre Site: Nothing to Report

**c. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

University of Minnesota Site: Nothing to Report

Hauptman Woodward Institute Site: Nothing to Report

Vancouver Prostate Centre Site: Nothing to Report

**6. PRODUCTS**

**a. Publications, conference papers, and presentations**

University of Minnesota Site: Nothing to Report

Hauptman Woodward Institute Site: Nothing to Report

Vancouver Prostate Centre Site: The following papers are relevant to either our in silico pipeline for drug-development or to VPC-14449 as a model drug to assist on other related projects.

1. Tam, K., Dalal, K., Hsing, M., Cheng, C.W., Chiang, Y.T., Sharma, A., Peacock, J.W., Wang, W., Cherkasov, A., Rennie, P.S., Gleave, M.E., and Ong, C.J. (2016) Androgen Receptor transcriptionally regulates Sema 3C in a GATA2-dependent manner. Oncotarget. Accepted.
2. Butler, M., Roshan-Moniri, M., Hsing, M., Lau, D., Kim, A., Yen, P., Mroczek, M., Nouri, M., Lien, S., Axerio-Cilies, P., Dalal, K., Yau, C., Ghaidi, F., Guo, Y., Yamazaki, T., Lawn, S., Gleave, M., Gregory-Evans, C., McIntosh, L., Cox, M.E., Rennie, P., and Cherkasov, A. (2016) Discovery and characterization of small molecules targeting the DNA-binding ETS domain of ERG in prostate cancer. Oncotarget. Accepted.
3. Dalal, K., Munuganti, R., Morin, H., Lallous, N., Rennie, P.S., and Cherkasov, A. (2016) Drug-discovery Pipeline for Novel Inhibitors of the Androgen Receptor. Meth. Mol. Biol. 1443:31-54.

**7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

**a. Individuals who have worked on the project:**

**University of Minnesota Site (Dehm, PI)**

Name	Dr. Scott Dehm
Project Role	PI
Researcher Identifier	orcid.org/0000-0002-7827-5579
Person months worked	1
Contribution to project	Dr. Dehm provided project oversight, supervised research and data analysis carried out by Drs. Che and Yang, secured regulatory approvals, managed grant budget and reporting, and coordinated with co-PIs Rennie and Gewirth.

Funding support	
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Name	Dr. Meixia Che
Project Role	Research Associate
Researcher Identifier	
Person months worked	7
Contribution to project	Dr. Che collected and analyzed data under supervision of Dr. Dehm
Funding support	

Name	Dr. Rendong Yang
Project Role	Research Associate (Bioinformatics)
Researcher Identifier	
Person months worked	1
Contribution to project	Dr. Yang provided bioinformatics support and planned ChIP-seq experiments
Funding support	

Name	Dr. Christine Henzler
Project Role	Research Associate (Bioinformatics)
Researcher Identifier	
Person months worked	2
Contribution to project	Dr. Henzler provided bioinformatics support and planned ChIP-seq experiments
Funding support	

### **Hauptman Woodward Institute Site (Gewirth, PI)**

Name	Dr. Daniel Gewirth
Project Role	PI
Researcher Identifier	Orcid.org/0000-0003-2702-3731
Person months worked	2
Contribution to project	Dr. Gewirth carried out oversight of the project, provided guidance and consultation to Dr. Que, and assisted with the collection of diffraction data.
Funding support	

Name	Dr. Nanette Que
Project Role	Staff scientist
Researcher Identifier	
Person months worked	6
Contribution to Project	Dr. Que carried out the protein purification and crystallization trials, collected preliminary diffraction data, and carried out ITC binding experiments
Funding support	

**Vancouver Prostate Centre Site (Rennie, PI)**

Name	Dr. Paul S. Rennie
Project Role	PI
Researcher Identifier	
Person months worked	2.4
Contribution to project	Dr. Rennie provided project oversight, and supervised research carried out by Dr. Dalal for the characterization of derivatives using biochemical assays.
Funding support	

Name	Dr. Artem Cherkasov
Project Role	Collaborator
Researcher Identifier	
Person months worked	2.4
Contribution to project	Dr Cherkasov oversees all aspects of computational drug design, molecular modeling and bioinformatics.
Funding support	

**b. Changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period:****University of Minnesota Site (Dehm, PI)**

The following grants have been activated since negotiation and setup of the current award:

Mayo Clinic Rochester (PI: Dehm) 07/31/2015-07/31/2017 1.08  
 CM (9%)  
 Prime: Prostate Cancer Foundation  
 Targeting aberrant AR and AR-V expressions and activity to overcome therapy resistance in castration-resistant prostate cancer  
 Mayo Administrative Contact: Suzanne M Taylor  
 Mayo Financial Contact: Susan Uhlenkamp, Research Finance Chair  
 Goals: The goal of this project is to develop antisense technologies to extinguish expression and activity of all forms of the full-length AR and truncated AR variants in prostate cancer.  
 Aims: I will be responsible for conduct of Aim 2, wherein TALEN-based gene editing will be used to develop AR- and AR-V-driven prostate cancer models with impaired function of PSA enhancer RNA (eRNA).

**Hauptman Woodward Institute Site (Gewirth, PI):** Nothing to Report

**Vancouver Prostate Centre Site (Rennie, PI):** Nothing to report

## **8. SPECIAL REPORTING REQUIREMENTS**

This report is for a COLLABORATIVE AWARD, and was prepared jointly by the three study PIs. The tasks are clearly marked with the responsible PI and research site.

## **9. APPENDICES**

NONE